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Forest  
Service

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# **Bacteria Monitoring in Shackleford and Mill Creek Grazing Allotments June 6 to September 24, 2013**



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## Background and Regulatory Context

In the summer of 2013 the Klamath National Forest (KNF) monitored in-stream fecal coliform bacteria to meet the range monitoring requirements of the Waiver of Discharge Requirements (NCRWQCB, 2010). Direction on how fecal bacteria must be sampled is specified in section 4 of the Waiver Monitoring and Reporting Program (NCRWQCB, 2012):

4.A.4: At each FIB monitoring site, USFS will collect samples for fecal indicator bacteria analyses within the high-use recreation area water during the grazing season at intervals sufficient to determine compliance with basin plan objectives. Standard sampling methods and commercial labs will be used.

4.A.5: If Basin Plan Objectives are exceeded, USFS will collect additional samples upstream and downstream of the high-use recreation area to isolate influences of humans, livestock, and other possible sources.

The Basin Plan objective for bacteria has three parts (NCRWQCB, 2011 section 3-4.00):

“The bacteriological quality of waters of the North Coast Region shall not be degraded beyond natural background levels.”

“In waters designated for contact recreation (REC-1), the median fecal coliform concentration based on a minimum of not less than five samples for any 30-day period shall not exceed 50/100 ml,”

“nor shall more than ten percent of total samples during any 30-day period exceed 400/100 ml.”

## Methods

Most of the streams and sample sites in the KNF monitoring program were established in 2011 as part of a larger water quality study conducted by the Rangeland Department at the University of California Davis (Roche, et al, 2013). We had to make changes to the U.C. sampling design in order to determine compliance with the North Coast Basin Plan objectives for bacteria. Two streams in active grazing allotments were selected including East Boulder Creek in the Mill Creek range allotment, and Shackleford Creek in the Shackleford range allotment (Figures 1 and 2). Kidder Creek was added as an ungrazed control where the allotment has been vacant since 2006 (Figure 3). A single site in Fox Creek immediately adjacent to Boulder Creek was added as an additional ungrazed control. All four streams are tributaries to the Scott River which is a tributary to the Klamath River. The allotments are located in the Marble Mountain and Trinity Alps Wilderness Areas and have relatively high recreation use with camping along the stream banks and swimming in Campbell and East Boulder Lakes. The North Coast Basin Plan has designated all four streams as supporting REC-1 contact recreation uses (NCRWQCB, 2011).

A total of 15 sites were selected in Shackleford Creek, 8 sites in Boulder Creek, 3 sites in Kidder Creek, and one site in Fox Creek. Sample sites were selected to isolate key grazing areas, typically above and below pastures, and below recreational use areas such as campgrounds, stock corrals, campsites, and lakes (Table 1). Observations of potential bacteria sources upstream from each site were made at the time the samples were collected. The sampling crew recorded the type of source observed such as deer or campers, the concentration of use such as a single animal or a whole herd, and whether the use was

recent or from previous years. The field sampling protocol is described in the Appendix. Duplicate samples and field blanks were taken at 15% of the total samples and one lab blank analyzed per sampling date. All samples were analyzed at the Quartz Valley Indian Reservation's state certified bacteria laboratory using IDEXX Collilert/Quanity Trays, following Standard Method number 9223 Enzyme Substrate Coliform Test (USEPA, 2009). Sample data is expressed in MPN/100ml, which is the most probable number of colony forming units per 100 ml sample.

Compliance with the first part of the Basin Plan standard for bacteria was evaluated using a before-after control-impact study design. Sampling was divided into two 30-day sampling periods before and after cows came on the allotments. At each site 5 samples were taken in June before grazing, and then another 5 samples repeated at the same sites in September after the allotments had been grazed for 2 months. Fecal coliform concentrations before grazing and in the control at Kidder Creek were used to identify background conditions, which were compared with the data from September to determine if bacteria had been degraded. Compliance with the second part of the Basin Plan standard was evaluated by comparing the median of the 5 samples in September with the numeric standard of 50/100ml. The third part of the Basin Plan standard was evaluated by comparing the percentage of total samples exceeding 400/100 ml in September to the numeric standard of 10 percent.

## Results

A total of 193 bacteria samples were measured in 2013. We were unable to collect 5 samples before the cows came on at Shackleford #3 and #18 so these sites were omitted from the before vs. after analysis. The 31 duplicate samples had an average difference of 18 MLN/100ml. All of the lab blanks were free of any fecal coliform, but 4 of the field blanks had fecal contamination between 6 and 53 MLN/100ml. The field crews thought the likely source was contaminated deionized water used in the blanks which was immediately discarded.

In the Shackleford allotment 80 cow-calf pairs were turned out on July 15 and removed between September 25 and October 1. The Mill Creek Allotment turned out 165 pairs on July 6 and removed them between September 28 and October 5. A comparison of bacteria before and after grazing shows that fecal coliform increased by an average of 200 MLN/100ml after grazing (Figure 4). Over the same period, the fecal coliform in the control streams increased by an average of only 7 MLN/100ml. No sites exceeded the Basin Plan standard for median fecal coliform before grazing, but 4 of 5 sites in Boulder Creek and 4 of 11 sites in Shackleford Creek exceeded the standard after grazing (Table 2). None of the control sites in Kidder or Fox Creeks exceeded the standard at any time. A t-test showed that 6 grazed sites had significantly higher fecal coliform than the background in control streams, and 4 sites had higher fecal coliform than the background measured before grazing (Table 3).

The percent of total samples exceeding the Basin Plan standard of 400/100 ml was calculated for the two 30-day periods before and after grazing (Table 4). Before the allotments were grazed, none of the samples in any stream exceeded 400/100ml. After the allotment was grazed Boulder Creek exceeded the Basin Plan standard but Shackleford Creek did not.

The ungrazed control in Fox Creek had a huge spike to 1300 MLN/100ml on September 3 that returned to its background level the following week (Figure 5). A campsite located 50 feet upstream from the sample site is the apparent source of bacteria. Similar campsites were observed at MC5, SF16, SF18, and SF19 that likely contribute to the large spikes at these sites. However, most of these sites also have

observed high use by cattle (Table 2) with chronically high bacteria levels that persisted for weeks after cows were turned out.

Many sites in Boulder and Shackleford Creeks had a spike in fecal coliform the week of June 25 to July 2 before cows came on (Figure 5). At Boulder sites 4, 6, and 8 fecal coliform spiked to over 98 on June 25 and then returned to between 1 and 28 the following week. At Shackleford sites 8, 10, and 16 fecal coliform spiked to over 300 on July 2. The sudden and widespread increase in fecal coliform coincides with a summer thunderstorm and rainfall event from 6/25 to 7/2 (Figure 6).

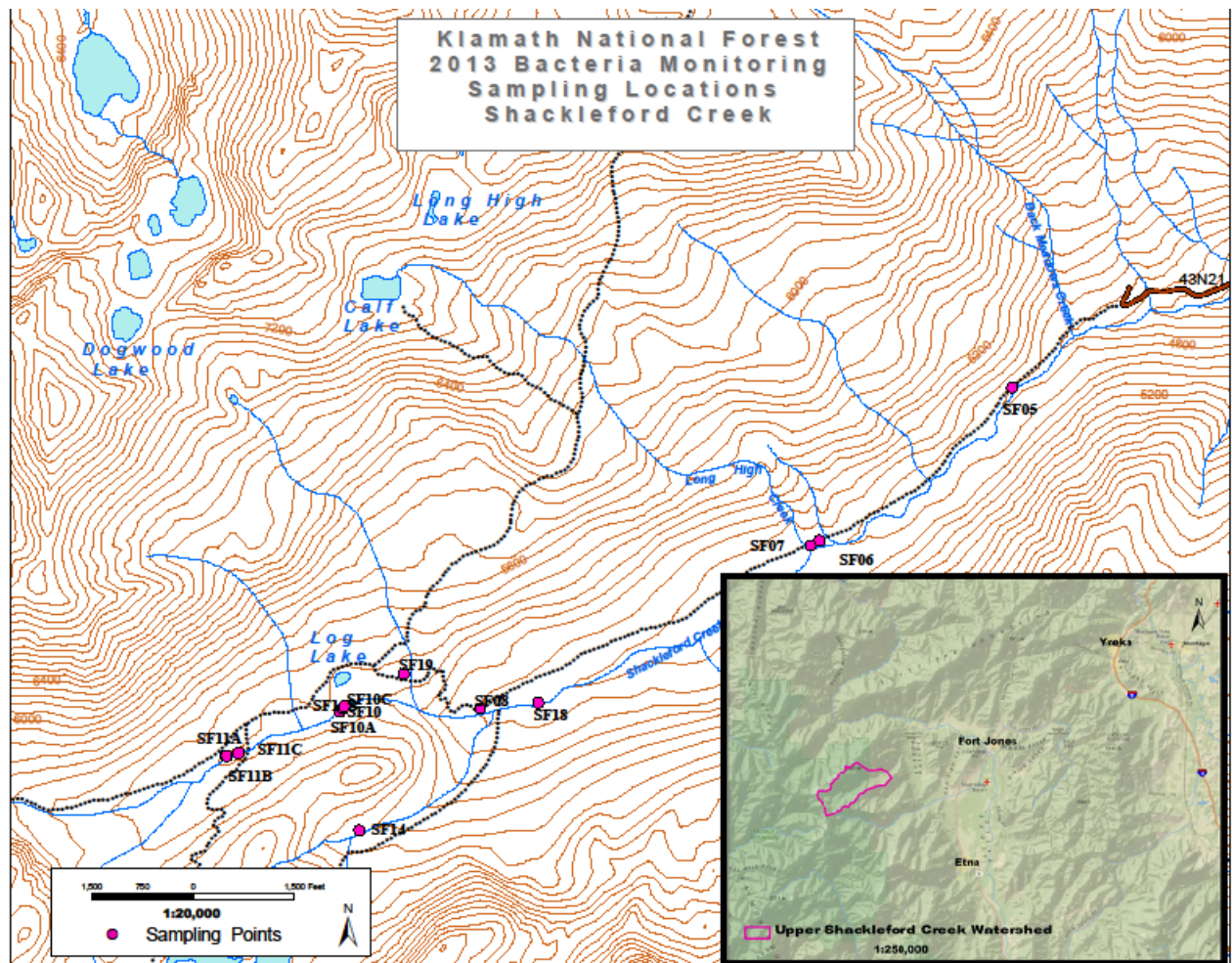


Figure 1. Sample sites in Shackleford Creek.

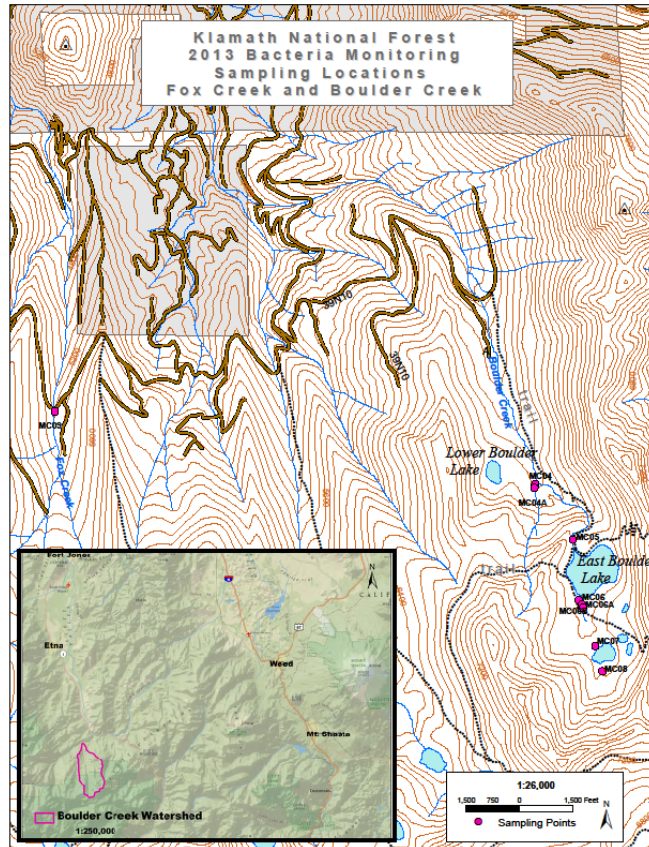


Figure 2. Sample sites in Boulder Creek (Mill Creek Allotment).

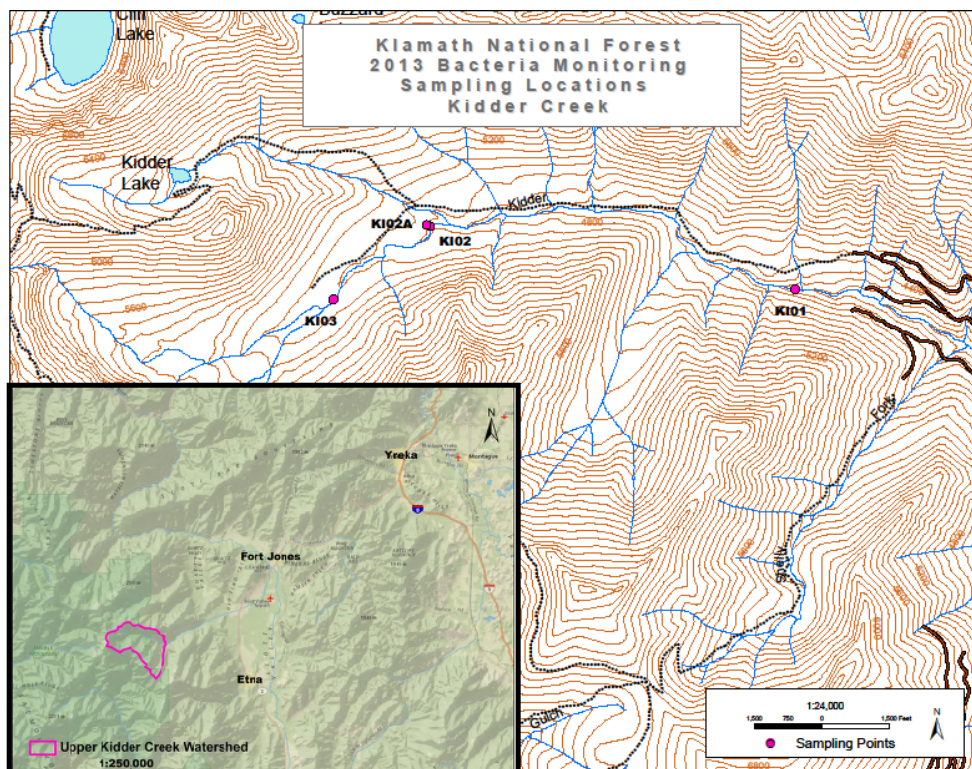


Figure 3. Sample sites in Kidder Creek, ungrazed since 2006.

Table 1. Bacteria sampling sites. All of these sites were established in Ken Tate's 2011 study except for Kidder Creek. Several sites were moved about 60 ft. to avoid low flows and stagnant water. GPS points in NAD 83, 10T, UTM's.

Site #	Stream	Easting	Northing	Description
MC04	Boulder	0517590	4565188	Unnamed tributary just above confluence with East Boulder Creek
MC04A	Boulder	0517585	4565158	Unnamed tributary just above confluence with East Boulder Creek; moved 75 ft. upstream on 8/27/13
MC05	Boulder	0517913	4564713	East Boulder Creek at the outlet of East Boulder Lake, below meadow complex
MC06	Boulder	0517962	4564198	East Boulder Creek above inlet for East Boulder Lake
MC06A	Boulder	0517991	4564157	East Boulder Creek above inlet for East Boulder Lake; moved upstream 30-40 ft. 7/2/13
MC06B	Boulder	0518001	4564132	East Boulder Creek above inlet for East Boulder Lake; moved 50 ft. upstream 8/27/13
MC07	Boulder	0518106	4563802	East Boulder Creek below outlet for Upper Lake
MC08	Boulder	0518166	4563588	East Boulder Creek above inlet for Upper Lake
MC09	Fox	0513486	4565802	Fox Creek at road, no grazing in this drainage
SF03	Shackleford			Shackleford Creek below campground and corral at end of Rd 43N21. No pregrazing samples were taken.
SF05	Shackleford	0494702	4600638	Shackleford Creek at gate below meadow complex, directly below rock wall
SF06	Shackleford	0493834	4599949	Long High creek above confluence with Shackleford Creek and above trail crossing
SF07	Shackleford	0493795	4599926	Shackleford Creek above confluence with Long High Creek
SF08	Shackleford	0492306	4599193	Shackleford Creek above Campbell Lake tributary (directly above log crossing)
SF10	Shackleford	0491690	4599198	Shackleford Creek below Log Lake Meadow complex and slightly above Lake
SF10A	Shackleford	0491674	4599179	Shackleford Creek below Log Lake Meadow complex and slightly above Lake; moved 60 ft. upstream 6/18/13
SF10	Shackleford	0491688	4599193	Shackleford Creek below Log Lake Meadow complex and slightly above Lake; moved 8/28/12
SF10C	Shackleford	0491694	4599203	Shackleford Creek below Log Lake Meadow complex and slightly above Lake; moved 60 ft. upstream 9/17/13
SF11A	Shackleford	0491158	4598981	Shackleford Creek in Log Lake Meadow, below trail junction
SF11B	Shackleford	0491162	4598979	Shackleford Creek in Log Lake Meadow, below trail junction; moved 50 ft. downstream, 8/28/12
SF11B	Shackleford	0491217	4598993	Shackleford Creek in Log Lake Meadow, below trail junction; moved downstream 30 ft. 9/4/12
SF14	Shackleford	0491761	4598644	Campbell Lake Trib below Campbell lake (swimming and dispersed camping) and above confluence with Unnamed Trib
SF16	Shackleford	0491032	4597828	Campbell Lake tributary above inlet for Campbell Lake
SF18	Shackleford	0492568	4599219	Isolates Emerald Tributary. Originally thought to be ungrazed but cows were observed near the site.
SF19	Shackleford	0491962	4599349	Isolates unnamed tributary above confluence with Shackleford Creek, near trail split for Calf Lake
KI01	Kidder	0495415	4595163	Kidder Creek above first unnamed tributary crossing Kidder Creek Trail from the wilderness boundary
KI02	Kidder	0492936	4595593	Kidder Creek above Kidder Lake tributary
KI02A	Kidder			Kidder Creek above Kidder Lake tributary;; moved 10 ft. upstream on 8/27/13 due to low flow
KI03	Kidder	0492281	4595095	Kidder Creek adjacent to Hays Meadow

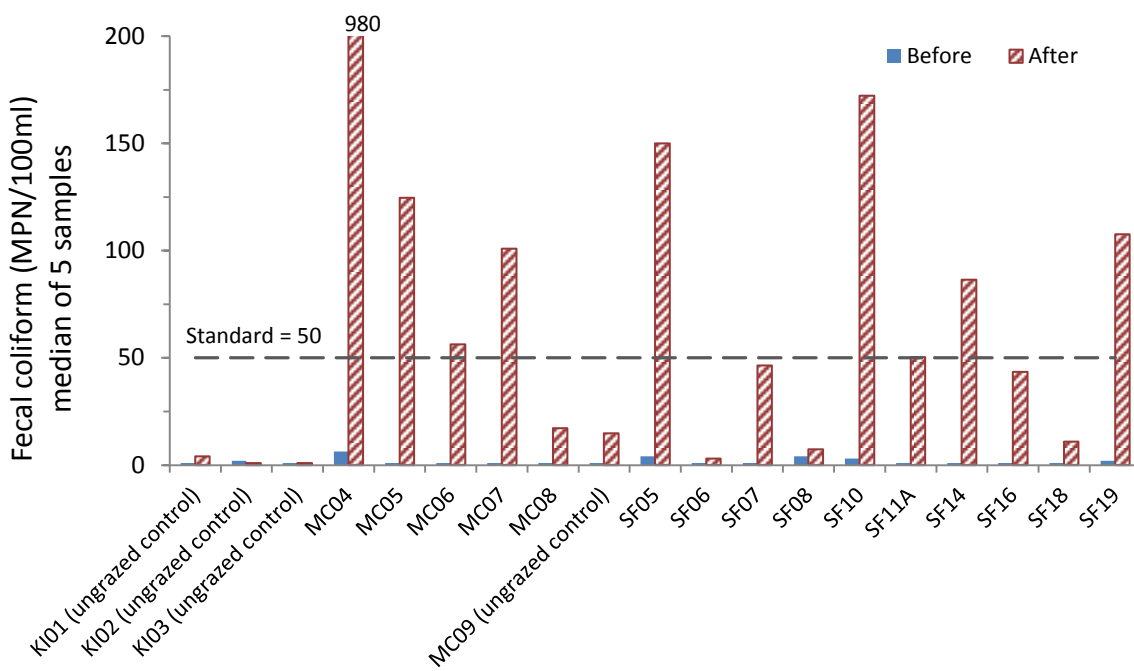


Figure 4. Median fecal coliform before and after grazing.

Table 2. Median fecal coliform concentrations for 30-day periods before and after grazing. Values in bold exceed the Basin Plan standard.

Stream (Site No.)	Control or Grazed	Before Grazing (June)		After Grazing (Sept)		Basin plan standard (MPN/100ml)	Observed Sources Before/After
		Median fecal coliform (MPN/100ml)	n	Median fecal coliform (MPN/100ml)	n		
Kidder (KI01)	Control	1	5	4	5	50	None / None
Kidder (KI02)	Control	2	5	1	5	50	Horses / None
Kidder (KI03)	Control	1	5	1	5	50	Horses / None
Boulder (MC04)	Grazed	6	5	<b>980</b>	5	50	Recreation / Cows
Boulder (MC05)	Grazed	1	5	<b>125</b>	5	50	Deer, Campers / None
Boulder (MC06)	Grazed	1	5	<b>56</b>	5	50	Recreation / Horses
Boulder (MC07)	Grazed	1	5	<b>101</b>	5	50	Recreation / Deer, Cows
Boulder (MC08)	Grazed	1	5	17	5	50	Recreation / Cows
Fox (MC09)	Control	1	5	15	5	50	Campers / None
Shackleford (SF03)	Grazed	na	0	11	5	50	None / Cows, Wildlife, Recreation
Shackleford (SF05)	Grazed	4	5	<b>150</b>	5	50	None / Cows
Shackleford (SF06)	Grazed	1	5	3	5	50	None / Cows
Shackleford (SF07)	Grazed	1	5	47	5	50	None / Cows
Shackleford (SF08)	Grazed	4	5	7	5	50	None / None
Shackleford (SF10)	Grazed	3	5	<b>172</b>	5	50	None / None
Shackleford (SF11A)	Grazed	1	5	50	5	50	None / Cows
Shackleford (SF14)	Grazed	1	5	<b>87</b>	5	50	Horses, Recreation, Wildlife / None
Shackleford (SF16)	Grazed	1	5	44	5	50	Campers, swimming, mules, elk and bear scat / Cows
Shackleford (SF18)	Grazed	1	3	11	5	50	None / Cows
Shackleford (SF19)	Grazed	2	5	<b>108</b>	5	50	Campers, horses / Cows

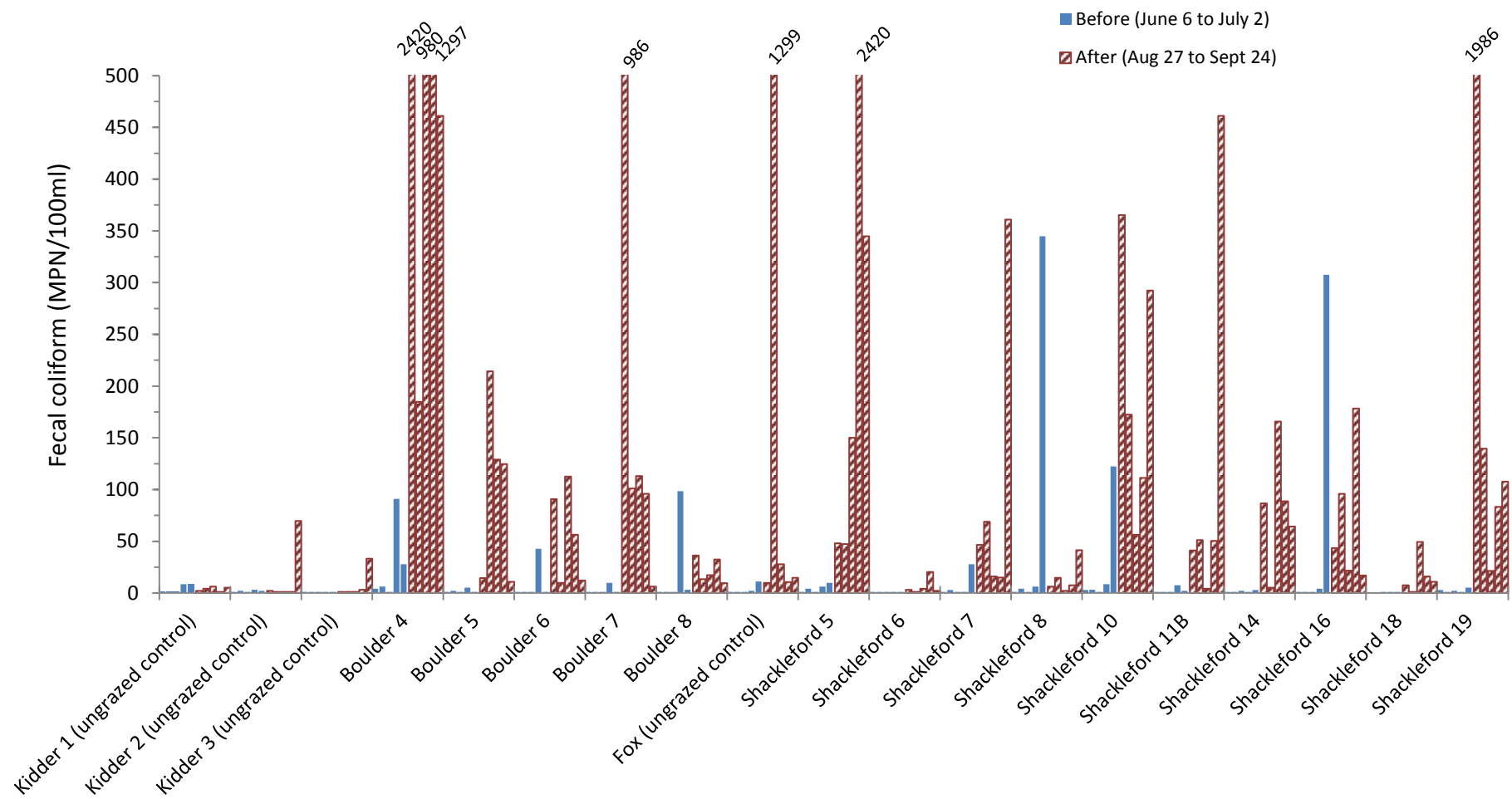


Figure 5. Fecal coliform measurements in chronological order at each site. Samples were taken at 7-day intervals for a total of 5 samples in June (blue) and 5 in September (red).

Table 3. Probability that fecal coliform in grazed streams was the same as the background level in Kidder Creek or before cows came on the allotment. T-tests for sites in bold indicate bacteria levels greater than background at a 0.05 significance level.

Site	DF	Grazed vs. Kidder (Unpaired, one-tailed)	Before vs. After (Paired, one-tailed)
Kidder (KI01)	4	na	0.448
Kidder (KI02)	4	na	0.196
Kidder (KI03)	4	na	0.170
Boulder (MC04)	4	<b>0.026</b>	<b>0.028</b>
Boulder (MC05)	4	<b>0.040</b>	<b>0.033</b>
Boulder (MC06)	4	<b>0.041</b>	0.051
Boulder (MC07)	4	0.120	0.116
Boulder (MC08)	4	<b>0.049</b>	0.481
Fox (MC09)	4	0.181	0.177
Shackleford (SF03)	na	na	na
Shackleford (SF05)	4	0.132	0.131
Shackleford (SF06)	4	0.327	0.114
Shackleford (SF07)	4	0.116	0.096
Shackleford (SF08)	4	0.269	0.203
Shackleford (SF10)	4	<b>0.014</b>	<b>0.015</b>
Shackleford (SF11A)	4	0.129	0.118
Shackleford (SF14)	4	<b>0.023</b>	<b>0.018</b>
Shackleford (SF16)	4	0.054	0.461
Shackleford (SF18)	na	na	na
Shackleford (SF19)	4	0.147	0.144

Table 4. Percent of total samples exceeding 400/100 ml for two 30-day periods before and after grazing. Bold exceed the Basin Plan standard of 10 percent.

Stream	Before Grazing (June)			After Grazing (Sept)		
	n	Number of samples	Percent of samples	n	Number of samples	Percent of samples
		exceeding 400/100ml	exceeding 400/100ml		exceeding 400/100ml	exceeding 400/100ml
Kidder (ungrazed control)	14	0	0.0	15	0	0
Boulder	30	0	0.0	30	6	<b>20</b>
Shackleford	48	0	0.0	50	3	6

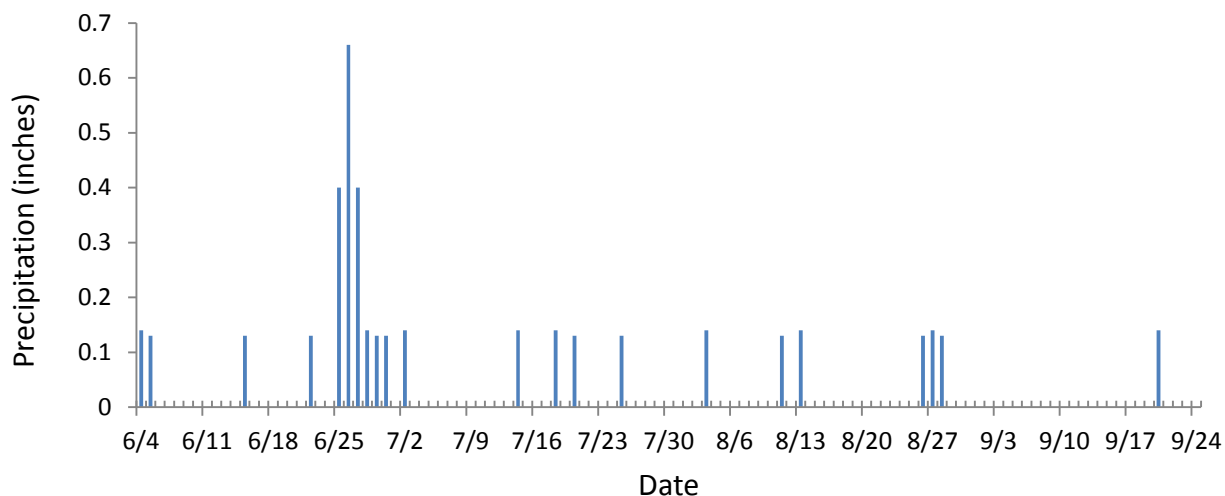


Figure 6. Precipitation at Scott Mountain (Bureau of Reclamation). Rainfall event from 6/25 to 7/2 coincides with a spike in fecal coliform in the 6/25 and 7/2 samples before cows came on allotments.

## Literature Cited

- North Coast Regional Water Quality Control Board, 2010. Categorical Waiver of Waste Discharge Requirements for Nonpoint Source Discharges Related to Certain Federal Land Management Activities On National Forest System Lands In the North Coast Region. Order No. R1-2010-0029.
- North Coast Regional Water Quality Control Board, 2011. Water quality control plan for the North Coast Region. Chapter 3, Water quality objectives.
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- Roche L.M., Kromschroeder L., Atwill E.R., Dahlgren R.A., Tate K.W., 2013. Water Quality Conditions Associated with Cattle Grazing and Recreation on National Forest Lands. PLoS ONE 8(6): e68127. doi:10.1371/journal.pone.0068127
- U.C. California Rangeland Watershed Laboratory, 2011. Water Quality Study Summary Shackleford and Mill Creek Grazing Allotments, Klamath National Forest.
- U.S. Environmental Protection Agency, 2009. Analytical Methods Approved for Drinking Water Compliance Monitoring under the Total Coliform Rule.

## **APPENDIX: Sampling Protocol for 2013 Water Quality Monitoring Project**

With cooperation of USFS, QVIR, NCRC, Karuk Tribe

### **QVIR Lab Supplies needed:**

120 (includes 15 extra) sample bottles, quanti-trays, colilert, gloves, coolers, blue ice or other ice packs, first aid kits

### **Field Gear Checklist:**

Chain of Custody datasheet

Photo log

Field notebook for other observations

Clipboard

Pens

100ml sample bottles (SF 10, MC 8, KI 6)

One 250ml sample bottle to mix duplicate samples in the field

Distilled water for field blank in a 250ml bottle

GPS – check batteries!

Camera – check batteries!

Thermometer

Cooler with ice / blue ice

Nitrile gloves in ziplock bags

Labels for sampling bottles

Copy of sampling protocol

Copy of directions to sites/Map

Food, drinking water

First aid kit

*Turn on incubator at QVIR*

### **Sites to be sampled:**

**Shackleford:** control site 18. Also sites 16, 11A, 10, 8, 7, 5

**Mill:** Sites 8, 7, 6, 4; control site 9.

**Kidder:** three sites to be determined

All sites are from the 2012 Tate study, except the Kidder Creek sites and site 11A, which is just below site 11 from Tate's study.

### **Sampling Protocol:**

1. Check batteries for camera and GPS, and turn on the incubator before leaving for sampling
2. Determine duplicate and field blank locations prior to leaving for sampling. Use a dice or random number generator to randomly choose these sites.
3. The naming convention for sample site ID is as follows: fieldblank or duplicate, watershed, site number, date. For example: **SF05082812** or **DupMC01082812**
4. Fill out the top of the Chain of Custody form with observer names, date, thermometer, camera, gps, weather conditions. Also fill out sample site locations to be taken so that none are missed.

5. Walk upstream and begin sampling at the farthest point from the trailhead.
6. At each site, record sample site ID, time, water temp, GPS coordinates and photo numbers on the chain of custody form along with any observations noted – Do not leave the observations section blank, address cattle, stock and recreational use at each site.
7. Take 100 ml bacteria sample at each site.
  - a. Use latex gloves when handling bottles during sampling. Fingers contain contaminants such as nitrates. Bug repellents or sunscreen are particularly troublesome as contaminants. Once the gloves are on, be careful not to touch your face, the ground, or anything but the bottles.
  - b. The sample should be taken from flowing, not stagnant water, facing upstream positioned in the thalweg.
  - c. Be sure to immerse the bottle completely, 10 cm (4 inches) deep, with mouth of bottle pointing upstream, so no water flows over your hand into the bottle. Be sure the bottle does not get near the bottom of the stream where sediments can be disturbed. Water samples should be collected 6-12 inches below the water surface. Fill bottle, to the 100ml line indicated, on first immersion, pour off the excess and cap. Get as close to the line as possible without underfilling (the water line must be at or just above the 100ml line). Do not redunk. If too much water is poured off, redo sample with new 100 ml container.
  - d. Do not touch bottle mouth or inside of cap. Be careful not to contaminate the sample with surface film, contact with human skin, breathing in/on the bottle or cap, etc. If stream is too shallow to immerse bottle fully, collect as much as possible, being very careful not to touch the bottom. Note depth on field notes.
  - e. Collect one "duplicate" sample. Sample sites chosen for duplicate sampling are selected at random among sites sampled. When a duplicate sample is selected for the site, collect the sample in the 250 ml sampling bottle provided. Cap the bottle and thoroughly mix the sample then pour 100 ml each into two clean regular sized sampling bottles. Duplicates document precision of laboratory analysis.
  - f. Samples are analyzed in the QVIR Bacteria lab. Keep samples cool while transporting. Store under 10 °C until analysis, but do not freeze. The maximum holding time is 6 hours. An additional 2 hours are allocated for processing at the lab.
8. Label bottle with sample site ID, time, water temperature and sampler's initials. For example: **MC02082812 1324 13C JB**
9. Immediately place sample(s) into ice pack for transport back to the trailhead.
10. Take at least three photos at each site and record the photo numbers on the photo log and chain of custody form.
  - a. Take one photo from the sampling location looking upstream.
  - b. Take one photo from the sampling location looking downstream.
  - c. Take one photo from the bank looking at the sampling location.
  - d. If there are any other notable observations at the site, take a photo of that and record the number on the chain of custody form and in the comments section on the chain of custody or on the back side of the chain of custody.

11. If any site is not collected for any reason, note that it wasn't collected and the reason.
- 12. Be sure samples arrive at the lab within 6 hours of the time that the first sample was collected.**
13. Record any other notable observations throughout the day (those that don't occur at sampling locations or if there isn't enough room on the data form to describe the observations) on the back of the chain of custody form along with GPS locations and photo numbers for these observations. Also record photo numbers in the photo log. Be sure to communicate these to your supervisor upon return from the field.
14. Document hand-off of samples to QVIR or any other in-between person in the bottom right corner of the data sheet. Retain a copy of the chain of custody form before handing the samples off to the next person if possible.
15. Photos will be downloaded at the QVIR or NCRC's office and stored in files according to camera and date.
16. GPS points should be recorded and maintained by the owner of the GPS unit (QVIR or NCRC). The first week it is imperative the UTM's are recorded on the data sheet in NAD 83 for each sampling location. Each crew should have these points loaded onto the GPS unit during subsequent sampling events.
17. Crew should gather gear needed for the next week's sampling event, including sampling bottles, labels, nitrile gloves, and data forms if necessary.

